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Research Note

Identification of *Enterobacteriaceae* from Washed and Unwashed Commercial Shell Eggs

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ABSTRACT

To evaluate the effect of processing on the safety and quality of retail shell eggs, a storage study was conducted with unwashed and commercially washed eggs. This work demonstrated that commercial processing decreased microbial contamination of eggshells. To know which species persisted during storage on washed or unwashed eggs, *Enterobacteriaceae* isolates were selected and identified biochemically. For each of three replications, shell eggs were purchased from a commercial processing plant, transported back to the laboratory, and stored at 4°C. Once a week for 6 weeks, 12 eggs for each treatment (washed and unwashed control) were rinsed in sterile phosphate-buffered saline. A 1-ml aliquot of each sample was plated onto violet red bile glucose agar with overlay and incubated at 37°C for 24 h. Following incubation, plates were observed for colonies characteristic of the family *Enterobacteriaceae.* A maximum of 10 isolates per positive sample were streaked for isolation before being identified to the genus or species level using commercially available biochemical strips. Although most of the isolates from the unwashed control eggs belonged to the genera *Escherichia* or *Enterobacter,* many other genera and species were identified. These included *Citrobacter, Klebsiella, Kluyvera, Pantoea, Providencia, Rahnella, Salmonella, Serratia,* and *Yersinia.* Non-*Enterobacteriaceae* also recovered from the unwashed egg samples included *Xanthomonas* and *Flavimonas.* Very few washed egg samples were contaminated with any of these bacteria. These data provide useful information on the effectiveness of processing in removing microorganisms from commercial shell eggs.

Since the early 1970s, commercial shell egg processing operations have shifted toward in-line, automated, spray-type washers using detergents and sanitizers with water warmer than freshly laid eggs *(9, 12).* Current commercial production operations have permitted year-round production, eliminating the need to store eggs for long time periods *(20).* A great deal of information has been published on levels and types of bacteria associated with eggshell surfaces, but much of it is more than 40 years old, when processing conditions were significantly different from today's operations *(1–3, 5, 6).*

A study to determine the effectiveness of sanitation practices in southeastern U.S. commercial shell egg facilities was conducted in 2003 *(11).* While there were large differences in the levels of bacteria recovered from plant to plant, the plants were all similar in that their sanitation practices had no effect on either aerobic or *Enterobacteriaceae* populations associated with the egg contact surfaces sampled in the egg processing plants. A follow-up project was conducted to analyze unwashed and washed eggs from one of the commercial facilities included in the previous study. Four different populations were monitored from 0 to 10 weeks of storage. A fifth population, *Enterobacteriaceae,* was monitored from 0 to 6 weeks of storage. On the basis of the results obtained in the follow-up study, washing effectively reduced bacterial numbers on eggshell surfaces throughout the storage period *(10).*

In the current study, presumptive *Enterobacteriaceae* isolates obtained from the follow-up storage study were randomly selected and saved for further analysis. Biochemical tests were used to identify these isolates to the genus or species level. Work described in this paper was performed to demonstrate which species were eliminated by commercial washing procedures and those that were able to survive washing and extended refrigerated storage on washed and unwashed eggs.

MATERIALS AND METHODS

Eggs were aseptically collected from a commercial in-line processing plant on three separate days (replications 1, 2, and 3). Unwashed eggs were collected from the accumulator belt as they entered the plant from the layer houses. Washed eggs were collected after they had been placed into Styrofoam cartons. For either treatment, sufficient eggs were collected that 12 eggs could be analyzed weekly for six consecutive weeks. Eggs were stored at 4°C prior to analysis. Eggshell surfaces were sampled by aseptically placing an egg into a sterile Whirl-Pak bag, adding 10 ml of sterile phosphate-buffered saline, and shaking for 1 min. After each egg was removed and discarded, the rinsate was duplicate pour plated using violet red bile glucose agar for the detection and enumeration of *Enterobacteriaceae.*

All violet red bile glucose plates were overlaid with 5 ml of

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TABLE 1. *Identification of isolates collected from 84 eggshell surface rinses of unwashed and washed eggs plated onto violet red bile glucose agar during 6 weeks of storage for replication 1*

Week	Unwashed eggs[a]	Washed eggs
0	*Escherichia coli* (7) *Yersinia* spp. (1) *Providencia rettgeri* (1) *Providencia* spp. (2)	ND[b]
1	*Xanthomonas maltophilia* (1) *Citrobacter youngae* (1) *E. coli* (4) *Flavimonas oryzihabitans* (2) *Pantoea* spp. (1) *Enterobacter cloacae* (1)	ND
2	ND	ND
3	ND	ND
4	*E. cloacae* (3) *Rahnella aquatilis* (2) *Enterobacter* spp. (1) *Klebsiella* spp. (1)	ND
5	ND	ND
6	*Serratia* spp. (1)	ND

[a] Number in parentheses indicates the number of isolates identified.
[b] ND, none detected.

TABLE 2. *Identification of isolates collected from 84 eggshell surface rinses of unwashed and washed eggs plated onto violet red bile glucose agar during 6 weeks of storage for replication 2*

Week	Unwashed eggs[a]	Washed eggs
0	*Escherichia coli* (11) *Klebsiella pneumoniae* (1)	ND[b]
1	*E. coli* (3) *Enterobacter cloacae* (3) *Enterobacter sakazakii* (5) *K. pneumoniae* (11) *Enterobacter* spp. (1)	ND
2	*E. sakazakii* (1)	ND
3	*E. coli* (1)	
4	ND	ND
5	*E. coli* (4) *E. cloacae* (5) *Salmonella* (1)	ND
6	ND	ND

[a] Number in parentheses indicates the number of isolates identified.
[b] ND, none detected.

violet red bile glucose agar and incubated at 37°C for 18 to 24 h. Plates with typical presumptive colonies were counted, and up to 10 colonies per positive sample were selected for subsequent identification. For samples with more than 50 colonies, a grid and a random number table were used for isolate selection *(19).* Each isolate was consecutively streaked three times on plate count agar plates and incubated overnight at 37°C to ensure clonality. After the third streak, a cultural suspension using 5 ml of physiological saline was prepared from each isolate. This material was used to inoculate API 20E strips (bioMérieux, Inc., Marcy l'Etoile, France). Strips were inoculated, incubated, handled, and analyzed according to manufacturer instructions. Reactions were recorded, and identifications were determined using Apilab Plus software (bioMérieux).

RESULTS AND DISCUSSION

The *Enterobacteriaceae* isolates described in this study were recovered from eggs previously described by Jones et al. *(11).* In that presentation, aerobic bacteria, yeasts/molds, *Pseudomonas* spp., and *Enterobacteriaceae* levels were monitored over time and statistically analyzed. Washed eggs had significantly fewer numbers of *Enterobacteriaceae* than did unwashed eggs for all sampling periods from weeks 0 to 6. Similarly, *Enterobacteriaceae* isolates were found less often on washed eggs than on unwashed eggs during the 6 weeks of observation. To increase the value of the data discussed in that paper, isolates were randomly selected and identified to genus or species. There have been other published studies on the types of bacteria isolated from shell eggs. However, most of these studies were either performed prior to the Egg Inspection Act being passed, or the bacteria were characterized more generally, i.e., by group or genus only. None of these studies focused on the progression of species during refrigerated storage *(9, 17).*

Enterobacteriaceae is a family of gram-negative, facultatively anaerobic rods that are associated with animal or plant hosts. There are more than 30 genera in this family of bacteria, including many human pathogens such as *Escherichia, Klebsiella, Salmonella, Shigella,* and *Yersinia (8, 13).* These bacteria are sometimes used to evaluate the ''sanitary'' or ''hygienic'' quality of raw foods *(14)* or to determine the hygienic conditions present during food processing. In most raw foods, they are not useful as an index of pathogen contamination *(13).*

Many types of organisms can be found on the shells of eggs, and they vary according to circumstances, though many are commonly found in air, soil, and water *(18).* In 1938, Haines *(6)* found that 38% of the microorganisms recovered from eggshells were non–spore-forming rods. Board et al. *(3)* have reported that *Escherichia* was present on most eggs but in small numbers, while *Aeromonas, Proteus,* and *Serratia* were recovered only occasionally. These researchers deduced that dust, soil, and feces were the major sources of eggshell contaminants. Florian and Trussell *(5)* correlated the presence of *Pseudomonas, Proteus, Escherichia,* and *Aerobacter* with black rot in eggs, *Proteus* with a custardlike rot, *Serratia* spp. with red rot, and *Pseudomonas* with pink rot.

In the present study, the genus and species determined using API 20E strips were tabulated for each of the three replications of unwashed and washed commercial shell egg surfaces (Tables 1 through 3). All isolates reported were identified with greater than 80% confidence. Identified isolates included *Escherichia coli, Enterobacter* spp., *Enterobacter cloacae, Enterobacter sakazakii, Serratia* spp., *Kluyvera* spp., *Salmonella, Citrobacter youngae, Klebsiella*

TABLE 3. *Identification of isolates collected from 84 eggshell surface rinses of unwashed and washed eggs plated onto violet red bile glucose agar during 6 weeks of storage for replication 3*

Week	Unwashed eggs[a]	Washed eggs
0	*Escherichia coli* (13) *Enterobacter cloacae* (1) *Enterobacter* spp. (1)	ND[b]
1	*Serratia* spp. (1)	ND
2	*E. coli* (9) *Kluyvera* spp. (1)	ND
3	*Rahnella aquatilis* (1)	ND
4	*E. cloacae* (1) *Enterobacter* spp. (1)	ND
5	ND	*Enterobacter amnigenus* (3) *Salmonella enterica arizonae* (1)
6	ND	ND

[a] Number in parentheses indicates the number of isolates identified.
[b] ND, none detected.

pneumoniae, Rahnella aquatilis, Providencia rettgeria, Providencia spp., *Yersinia* spp., *Pantoea* spp., *Xanthomonas maltophilia,* and *Flavimonas oryzihabitans.*

Isolation of *Citrobacter, Escherichia, Enterobacter, Klebsiella, Serratia,* and *Salmonella* from the shells of eggs was not surprising. These organisms have 37°C as their optimal growth temperature and are commonly isolated from the environment or from the intestinal tracts of vertebrate animals *(8).* In particular, *Salmonella* has been isolated from the avian species and their environments, including layer houses and egg processing plant environments *(4). Providencia, Kluyvera,* and *Rahnella* also grow optimally at 37°C but are isolated less often, particularly from humans. *Pantoea* and *Yersinia* are isolated from a wide variety of environmental and animal sources. These organisms grow optimally between 28 and 30°C but were isolated from media incubated at 37°C, which is within their growth range. The former genus contains two opportunistic pathogen species, while the latter includes a well-known foodborne pathogen *(8).*

E. sakazakii was recovered from eggshells at weeks 0, 1, and 2 in the first repetition. This species has been recovered from milk-based powdered infant formula products in several countries *(16)* and can cause meningitis, sepsis, or necrotizing enterocolitis in newborns fed contaminated products. Ultraheated milk, spoiled tofu, lettuce, fermented bread, and rinsed beer mugs have been source of *E. sakazakii.* This organism has been isolated from the guts of stable fly larvae *(7).* Perhaps flies are vectors in the layer house environment. None of the isolates identified from washed eggs were *E. sakazakii.* A recent study reported recovering this organism from eight of nine food processing plants and from 5 of 16 households *(15).*

Three of the species identified were non-*Enterobacteriaceae. F. oryzihabitans* is a gram-negative aerobic/microaerophilic rod often found in the general environment that is a saprophyte or commensal of humans and other warm-blooded animals. It occasionally proves to be pathogenic for humans. *X. maltophilia,* which grows optimally at 28 to 30°C, was recovered from a single sample. This organism is most often associated with plants but can be an opportunistic human pathogen *(8).*

E. coli was isolated during the week 0 sampling for all three replications but was also recovered at weeks 1, 2, 3, and 5 for at least one of the three repetitions. This organism was isolated more often than all other identified isolates combined (54 of 105) for all three replicates but was recovered only from unwashed eggshells. *Enterobacter* spp. was the second most frequently isolated genus.

There were far fewer isolates from washed eggs. *Enterobacter amnigenus* was the species isolated most often from washed eggs (three of four) but only at week 5 of the third repetition. The other isolate recovered was *Salmonella e. arizonae.* No washed eggshells were contaminated with *Enterobacteriaceae* within the advertised shelf life for retail eggs (about 30 days postpackaging). It seems more likely that these organisms survived on the surface of the egg rather than grew to detectable levels. Too few isolates were identified from washed eggs to draw definitive conclusions.

Commercial washing eliminated many species from the surface of table eggs. This work demonstrated the variety of species associated with the shells of unwashed eggs that were removed by commercial washing processes observed in a U.S. Department of Agriculture-Agricultural Marketing Service–inspected facility.

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